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AD_____

Award Number: DAMD17-98-1-8151

TITLE: Relationship between Pak-Mediated Cell Death and Stress-Activated Kinase Signaling in Breast Cancer

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CONTRACTING ORGANIZATION: The Scripps Research Institute
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REPORT DATE: February 2000

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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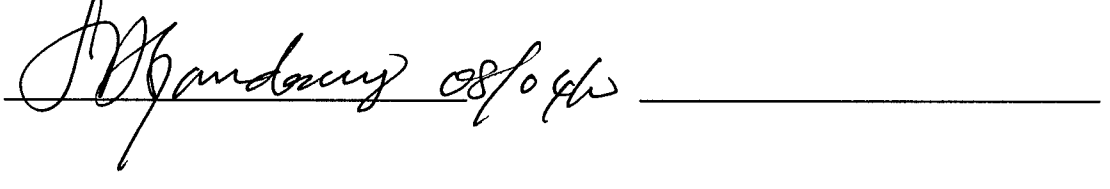

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	February 2000	Annual (1 Feb 99 - 31 Jan 00)

4. TITLE AND SUBTITLE
Relationship between Pak-Mediated Cell Death and Stress-Activated Kinase Signaling in Breast Cancer

5. FUNDING NUMBERS
DAMD17-98-1-8151

6. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
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12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

p21-activated kinases or Pak`s have been implicated in apoptosis as an upstream regulator with enhancing or suppressing influence and recognized as part of the cell death execution machinery. Here we show that a correlation exists in breast cancer cells between caspase-dependent cleavage of the Pak2 and activation of c-jun kinase. The mode of Pak kinase regulation has recently been identified. Small GTPases like Rac and Cdc42 or sphingoid-like lipids bind to Pak kinases, disrupt an intramolecular inhibitory interaction leading to increased phosphorylation of substrates. This intramolecular interaction is also disrupted by caspase-cleavage of Pak2. The autoinhibitory 83 amino acid region which interacts with the Pak kinase domain and inhibits its activity might allow us to specifically inhibit signaling pathways downstream of Pak and evaluate how the cell death process is affected. In a biochemical approach screening for substrates and possible mediators of cell death signaling components via Pak kinases we identified a guanine nucleotide exchange factor is phosphorylated by the Pak. In future studies we are going to evaluate the possibility of this GEF being a mediator of JNK and cell death signaling.

14. SUBJECT TERMS
Breast Cancer

15. NUMBER OF PAGES
24

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Limited

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Frank Lenk 2-24-00

PI - Signature Date

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INTRODUCTION

Programmed cell death is a means of the cellular machinery for self-elimination and plays an important role in development, tissue reorganization and the immune system. It is of great importance to understand this process on a molecular level and to identify components involved in the onset, suppression and execution of this form of cell death. The purpose of this study is to analyze the role of the pleiotropic protein kinase regulator Pak (p21-activated protein kinase) on signaling cascades leading to programmed cell death. Pak kinase is an established regulator of the cytoskeleton and MAPK signaling pathways and might influence cell death via these components. The Scope of this study is to identify the relationship between Pak kinase signaling and apoptosis which might exist via the stress-activated protein kinase signaling pathways.

BODY

In an initial approach we screened a variety of stimuli for their ability to induce programmed cell death in established breast cancer cell lines [technical objective 1, task1]. For this assay the cells were seeded in 35 mm dishes and incubated for 24 to 28 hours with the indicated stimuli (Table 1). Apoptosis was measured by a DNA laddering and apoptotic morphology was verified by light microscopic evaluation. As seen from the results, breast cancer cell lines vary markedly in their sensitivity towards different apoptotic stimuli (Table 1). We have not been able to induce apoptosis by serum withdrawal in all tested cell lines. Interestingly, the non-transformed breast epithelial cell line 184A1 was not sensitive to any of the apoptotic stimuli tested. As a preliminary hypothesis we conclude that the genetic background of the tested breast cancer cell lines might determine whether the cells are sensitive towards a specific stimulus or not. Mutations or deletions might be responsible for the observed differences between the breast cancer cell lines. As an example, it is known that the MCF-7 cells are not responding to Fas ligation perhaps due to a shift in Fas translocation from the membrane to cytosol; however the sensitivity of MCF-7 cells to Fas ligation can be restored by vitamine E succinate treatment which might lead to a translocation of Fas to the membrane (Yu et al., 1999)

Recently, it has been shown by our collaborators that certain breast cancer cell lines (T47-D, MCF-7, MDA-MB435) contain an activated form of the Rac3 GTPase, which in turn is responsible for the activation of the effector kinases Pak1/2 (Mira et al., 2000). Deactivation of the Rac3-GTPase using virally transfected dominant-negative Rac3 leads to inactivation of Pak1/2. Is was hypothesized that deregulation of the Rac3 GTPase is due to upregulation of Rac3 exchange factor activity.

In our laboratory it has recently been demonstrated that Pak1 kinase can phosphorylate the cell death agonist Bad, which leads to dissociation from the anti-apoptotic regulatrs Bcl-2/Bcl-Xl and hence suppression of the apoptotic response (Schurmann et al., 2000). Thus, Pak has a similar function as Akt and protein kinase A, i.e. to prevent cell death in response to growth factor signaling. However, the presence of activated Pak in these three cell lines does not correlate with the sensitivity towards any of the tested apoptotic stimuli.

In Jurkat cells we have demonstrated that Fas-induced apoptosis leads to an increase in c-jun phosphorylation (Rudel et al., 1998). To analyze the relationship between Pak2 cleavage and c-jun kinase activation [technical objective 1, task2] we stimulated T47-D cells with anti-Fas IgM antibodies and took samples for Pak2 detection and JNK activity assays at

various time points (Fig.1A). As seen in the autoradiograph, Fas-ligation leads to a detectable activation of JNK already after 2 hours and peaks at 6 hours. c-jun phosphorylation was 20-fold higher at the 6 hour time point than at time point zero or compared to non-stimulated control cells. Activation of JNK paralles the appearance of Pak2 cleavage products which we detected using polyclonal Pak-specific antibodies. Cleavage of Pak2 can be inhibited by the addition of caspase inhibitors YVAD-cmk and DEVD-ald to the medium (Fig.1B). YVAD-cmk led to complete inhibition, while DEVD-ald only partially inhibited Pak2 cleavage. The presence of the inhibitors also leads to inhibition of JNK signaling which is in line with our hypothesis that JNK activation is caspase-dependent. Again, the stringency of caspase inhibition – as seen for Pak2 cleavage- is reflected in JNK activation.

Recently, we have been able to characterize an 83 amino acid domain (hpak1$^{67-150}$) within Pak1 with autoinhibitory activity (Zenke et al., 1999). Addition of this domain to *in vitro* kinase assays demonstrated that auto- and substrate phosphorylation of GTPase- or sphingosine-stimulated Pak can be inhibited. The autoinhibitory region is conserved within the family of Pak kinases suggesting a general mode of intrasteric kinase regulation. We have been able to show Pak2 can be cleaved *in vitro* with recombinantly expressed caspase-3 and kinase activity gets significantly activated as measured by phosphorylation of myelin basic protein (Fig.2A,B) supporting our previous assumption that proteolytic cleavage of Pak2 is activating the kinase (Rudel and Bokoch, 1998). Caspase-3 mediated activation of Pak2 can also be efficiently inhibited by the characterized hpak1$^{67-150}$ peptide (Fig.2B), thus providing us with a valuable tool to assess the role of Pak2 cleavage *in vivo* during apoptosis [technical objective 2]. Interestingly, a bacterially expressed Pak2 C-terminal fragment (resembling the caspase-3 cleavage product) is is not sensitive to inhibition by the hpak1$^{67-150}$ (Fig.2B). Indirect evidence suggests that phosphorylation events within the kinase domain render it inactive towards inhibition (Zenke et al., 1999).

To specifically assess the role of caspase-mediated activation of Pak2 during apoptosis we designed an experimental setup to circumvent potential effects of the inhibitory domain during signaling processes upstream of caspase activation [technical objective 2]. It has been recently established that injection of cytochrome c can rapidly induce apoptosis in fibroblast and breast cancer cell lines (Zhivotovski et al., 1998). Cytochrome C injection presumably leads to activation of the initiator caspase-9 in conjunction with Apaf-1, regulatory components downstream of mitochondria in the apoptosis signaling cascade. In initial injection experiments, we could demonstrate that cytochrome C injection kills Swiss3T3 fibroblasts very efficiently within 2 hours (Fig.3). The cytochrome c mediated

death response is dependent on the activation of caspases since it is inhibited by coinjection with zVAD. We are currently investigating whether coinjection of the autoinhibitory domain impacts on the cell death response.

Transfection of the Pak2 kinase domain has been shown to induce cell death in Jurkat T lymphoblast cells. The cell death response was dependent on kinase activity since a kinase-inactive version of this domain had no cytotoxic effect. We wanted to analyze whether overexpression of the Pak2 kinase domain would have a cytotoxic influence on breast cancer cells [technical objective 2, task1]. The Pak2 kinase domain (amino acids 213-525) was co-transfected with a lacZ-expressing plasmid pCMV5lacZ. A potential cytotoxicity would therefore be visualized by cell rounding/blebbing of blue cells or – if the cell death response is more advanced- by a decrease in lacZ-positive cells on the culture dish. However, in initial transfection experiments we observed that the kinase-active Pak2 construct did not yield an active Pak2 protein as measured in *in vitro* kinase assays with myelin basic protein as a substrate. A similar phenomenon has been observed with a Pak1. We and others have indications that mammalian expression plasmid containing Pak1/Pak2 are prone to mutations during plasmid propagation possibly due to partial expression and cytotoxicity in *E.coli*. For full-length Pak1 it has been demonstrated by C.C. King in our laboratory that expression at 30°C can circumvent the cytotoxicity effect in *E.coli*. We are currently investigating if this strategy is useful for the propagation of expression vectors containing the Pak2 kinase domain.

In an approach to identify new substrates of Pak kinases we used the Pak1 kinase domain as a bait to pull down putative candidate proteins from crude lysates [technical objective 3]. A glutathione S-transferase fusion protein of the Pak1 kinase domain (GST-Pak1$^{233-544}$) was immobilized on glutathione-agarose beads and incubated with the lysates. After extensive washing steps with detergent-containing lysis buffer, the pulldown fraction was denatured and analyzed by SDS-polyacrylamide gelelectrophoresis. In initial experiments we used Jurkat T cells, T47-D and MCF7 breast cancer cell lysates in our binding experiments (Fig.4A). We were able to specifically pull down a protein of a molecular size of about 115 kDa which was not precipitated with glutathione S-transferase GST alone as a bait. This protein was especially pure and enriched in pulldown fractions from Jurkat cells (Fig.4A). For the matter of purity, we continued in our characterization using this cell type.

We tested the pulldown fraction containing GST-Pak1 and the bound 115 kDa protein in *in vitro* kinase assays with radiolabeled ATP. In these assays the 115 kDa protein was substantially phosphorylated (Fig.4B); it is remarkable that the activity of GST-Pak1 also signinicantly increased in comparison to GST-Pak1 that was not incubated with Jurkat lysate. Binding of proteins to Pak might therefore enhance enzymatic activity. In the presence of a recombinantly expressed autoinhibitory peptide ($hpak1^{67-150}$) phosphate incorporation into the Pak interacting protein was significantly reduced, demonstrating that Pak1 activity was responsible for phosphate incorporation into the substrate. Interestingly the autoinhibitory peptide was able to inhibit binding of Pak1 to the 115 kDa protein (data not shown) suggesting that the unknown 115 kDa protein and the autoinhibitory region bind to the same or an overlapping region in the kinase domain.

We used time-of-flight mass spectroscopy (MALDI-TOF) to identify the Pak-interacting protein. Pulldown fractions were electrophoresed by SDS-PAGE, the Coomassie Blue-stained protein was excised and the gel piece was incubated overnight with trypsin. Tryptic peptides were eluted from the gel, desalted and co-crystallized with 2,5 Di-hydroxybenzoic acid DHB („dried droplet method") on a MALDI sample holder plate. The peptide masses were determined using a Dynamo MALDI-TOF mass analyzer. The calculated peptide masses were used to search for proteins in the databases with a similar or identical mass fingerprint. 10 out of 20 peptide masses matched a protein named KIAA0651 which originates from a cDNA clone (HK01046) isolated from human brain.

The cDNA HK01046 encodes for a protein of 910 amino acides with a predicted molecular weight of 103 kDa. However, direct sequencing of RT-PCR products revealed the presence of a 68 nucleotide insertion at in the 5`-region of the cDNA, which generates a new N-terminal region 77 amino acids longer than the original HK01046 cDNA clone which then codes for a 987 amino acid protein of 112 kDa in size; however, the prediction for the ATG start codon is uncertain.

The protein sequence of KIAA0651 is about 90% homologous to GEF-H1, a microtubule binding guanine nucleotide exchange factor which has been isolated from HeLa cells (Ren et al., 1998). Both derived protein sequences share strong homology in their functional domains (Dbl-homology domain, microtubule-binding domain). In fact, the identity on the nucleotide level suggests that both open reading frames originate from the same genomic locus but represent splice variants; single base deviations, base deletions present in the nucleotide comparison are mostly due to sequencing errors as suggested by cDNA sequence information from EST databases (expressed sequence tags).

GEF-H1 contains a dbl homology domain and a microtubule binding domain and in its recent publication it has been demonstrated to contain both activities (Ren et al., 1998). Interestingly, this exchange factor has been shown to have activity towards Rho and Rac but not Ras or Cdc42. Ren et al. have produced a polyclonal antiserum against a protein region which is also present in KIAA0651. Using the antiserum in western blots we could show that the 115 kDa protein crossreacted with the antibody (Fig. 5).

α-GEF-H1 immunoprecipitates were compared with GST-Pak1 pulldown fractions with respect to their ability to become phosphorylated by Pak1. A protein band of the same size as in the GST-Pak1 pullldown fraction was phosphorylated by GST-Pak1 in *in vitro* kinase assays. We could show that full length human Pak1 immunoprecipitated from Cos-1 cells was also able to phosphorylate the Pak-interacting protein in the presence of activated Cdc42 (data not shown).

To get protein sequence information of the Pak-interacting protein we purified the protein for Edman degradation. Pulldown fractions were electrophoresed and blotted onto PVDF membranes and the membrane pieces were sent for amino-terminal microsequencing. However, the sequence information was highly ambiguous possibly due to a chemical block at the N-terminus. In future purifications we are trying to employ several precautions to prevent a chemical blockade of the protein. If conventional protein sequencing of the amino-terminus does not lead to a result we would like to sequence tryptic peptides or use a mass spectrometry-based approach to get partial sequence information.

The open reading frame of KIAA0651 has been amplified from a cDNA clone (HK01046, received from Kazusa DNA Reseach Corp.) and subcloned into a mammalian expression plasmid containing a hemagglutinin epitope. The constructed plasmid was transfected in Cos7-cells and the lysates were analyzed for protein expression. Lysates expressing KIAA0651 were then checked for protein interaction in the above described binding experiments using the Pak1 kinase domain. (Fig.6). We could demonstrate that KIAA0651 was specifically pulled down with Pak1.

In future experiments we want to explore whether the interaction with or the phosphorylation by Pak regulates the activity status and/or localization of KIAA0651. I have already established the guanine nucleotide exchange assay to measure the putative exchange activity of KIAA0651. GST-fusion proteins of RhoA, Rac1/2/3 and Cdc42 are purified from bacterial lysates. As controls for guanine nucleotide exchange factors we use dbl and lfc expressed in baculovirus insect cells.

We want to express KIAA0651 in Cos-cells alone or in combination with wildtype, constitutively activated or kinase-inactive Pak1 to assess the question if both proteins colocalize and are in a functional relationship with each other. We want to explore the possibility of KIAA0651 being a signaling mediator to JNK/p38 which could explain the onset of SAPK signaling in breast cancer cells stimulated to undergo apoptosis.

In summary, cleavage of Pak2 during apoptosis might be a crucial event leading to activation of the JNK signaling pathway. As we could demonstrate using T47-D breast cancer cells stimulated with anti-Fas IgM antibodies cleavage of Pak2 correlates with the activation of cjun kinase and activation is sensitive towards caspase inhibitors. Due to difficulties with the expression of the Pak2 kinase fragment we do not know up to now whether Pak2 activity itself is sufficient to mediate a death signal in breast cancer cells.

To isolate putative mediators of signaling components to SAPK we used a biochemical approach to identify substrates of Pak kinases. We have been able to identify a microtubule-binding Rho/Rac exchange factor which might represent a substrate of Pak important in signaling to SAPK kinases.

KEY RESEARCH ACCOMPLISHMENTS

- Sensitivity characteristics of various breast cancer cells towards different apoptotic stimuli

- Correlation between Pak2 cleavage and JNK activation in Fas-induced apoptosis

- Inhibition of caspase-cleaved and activated Pak2 by a Pak1 derived autoinhibitory peptide

- Establishment of a cytochromc injection model to study Pak2 effexts during apoptosis

- Identification of KIAA0651 as a binding partner and substrate of p21-activated kinase

REPORTABLE OUTCOME

King, C.C., **Zenke, F.T.**, Dutil, E.M., Newton, A.C., Hemmings, B.A., Knaus, U.G., Bokoch, G.M. Sphingosine–stimulated 3-phosphoinositide-dependent kinase-1 (PDK1) phosphorylates p21-activated kinase (Pak1). manuscript submitted

Zenke, F.T. and Bokoch, G.M. The role of caspase-mediated Pak2 activation. Keystone Symposium in Breckenridge, Colorado on programmed cell death, 4/6/99 to 4/11/99

Zenke, F.T., King, C.C. and Bokoch, G.M. Regulation of Pak activity by autoinhibition and phosphorylation. San Diego Cell Biology Meeting, 5/20/99.

CONCLUSIONS

Activation of Pak2 kinase by proteolytic cleavage is presumably responsible for caspase-mediated activation of SAPK kinases during the apoptotic response. We have presented evidence that the cell death response in T47-D breast cancer cells also involve an upregulation of JNK activity which is dependent on caspases. We identified KIAA0651 as a substrate of p21-activated kinase. This guanine nucleotide exchange factor might represent a missing link between Pak kinases and the JNK/p38 kinases and be an important mediator of a Pak-mediated cell death response. The identification of a microtubule-binding exchange factor as a Pak substrate opens a new perspective to cell death signaling by Pak kinases. Microtubule-stabilizing and/or depolymerizing drugs are long established chemotherapeutics used to eliminate cancer cells. Therfore it might be valuable to test if Pak is modulating the apoptotic sensitivity towards drugs disturbing the microtubule network. A possible relationship between microtubules and SAPK kinase signaling will be explored in this context.

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TABLE 1

cell line	control	α-Fas	TNFα/PDTC	C2-ceramide	VP16	serum-with-drawal
184A1	-	-	-	-	-	-
T47D	-	+	-	-	+	-
MCF-7	-	-	+	+	-	-
HS578T	-	+	-	-	-	-
ZR751	-	-	-	-	-	-
SKBR3[a]	-	-	+	-	+	-
MDA-MB231[a]	-	-	-	-	-	-

Cells were plated at 50% confluency in 35 mm dishes and incubated o/n in serum-containing complete medium. After 12-24 hours the medium was exchanged for complete medium containing the indicated stimuli. The concentractions of apoptotic stimuli used were: α-Fas: 150 ng/ml; TNFα/PDTC: 20 $ngml^{-1}$/10 μM; C2-ceramide: 50 μM; etoposide VP16: 30 μM

[a]: The backgroung level of detached and blebbing cells was remarkably high even in control cells. However, this did not correlate with significant DNA fragmentation as visualized by agarose gelelectrophoresis.

A

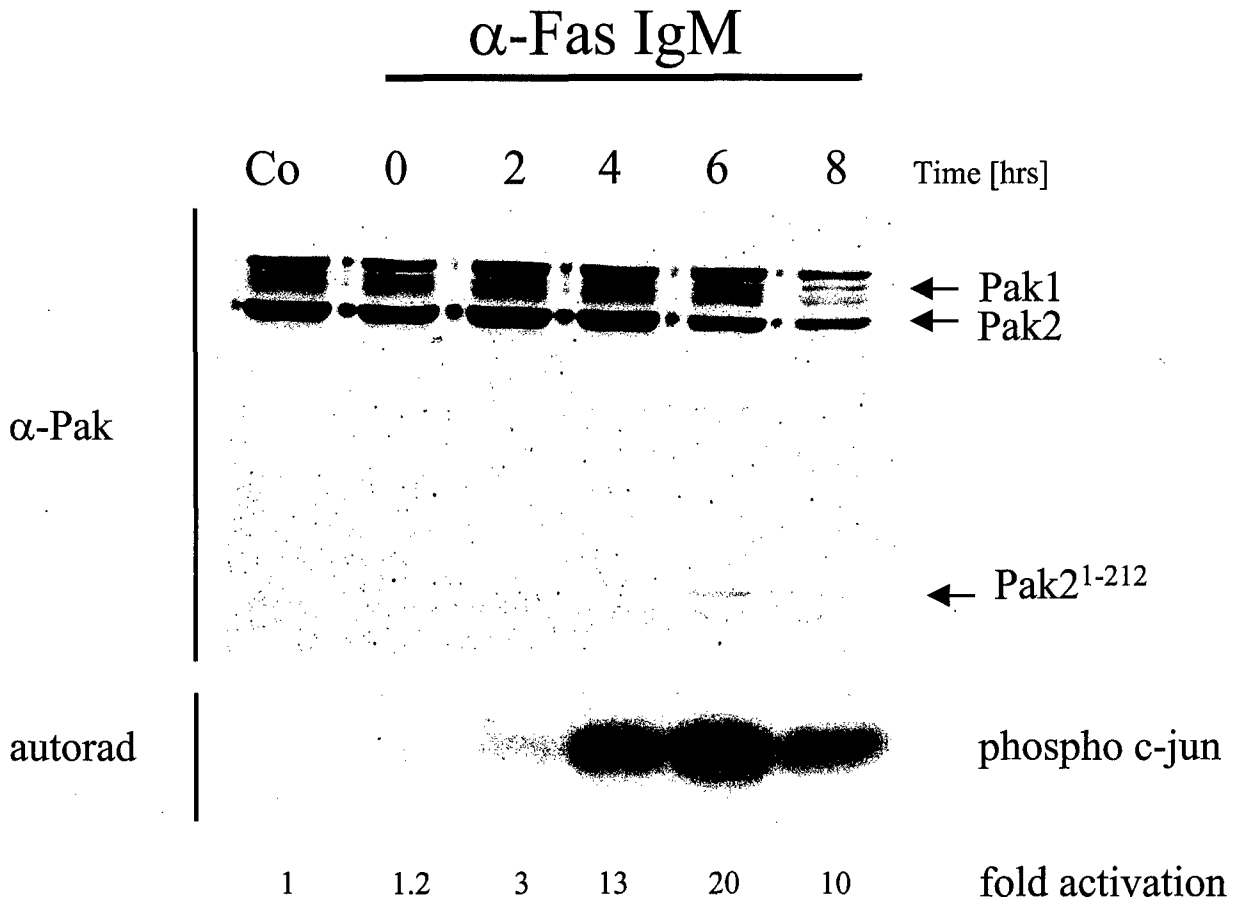

Figure 1A: **Fas ligation in T47-D breast cancer cells.** Breast cancer cells were plated onto 10 cm dishes, grown o/n and stimulated with anti Fas IgM antibody at 150 ng/ml for the indicated time points or left untreated (Co: control). Cells were lysed and the lysates were analyzed by western blotting using Pak-specific polyclonal antiserum (2125/5) detecting both Pak1 and Pak2 isoforms. Note the appearance of the N-terminal Pak2 cleavage product (upper panel). A fraction of the lysates were processed for the JNK activity assay (lower panel) using GST-cjun as a substrate. Incorporation of radiolabeled ATP was visualized by autoradiography and subsequently quantified by phosphorimager analysis.

B

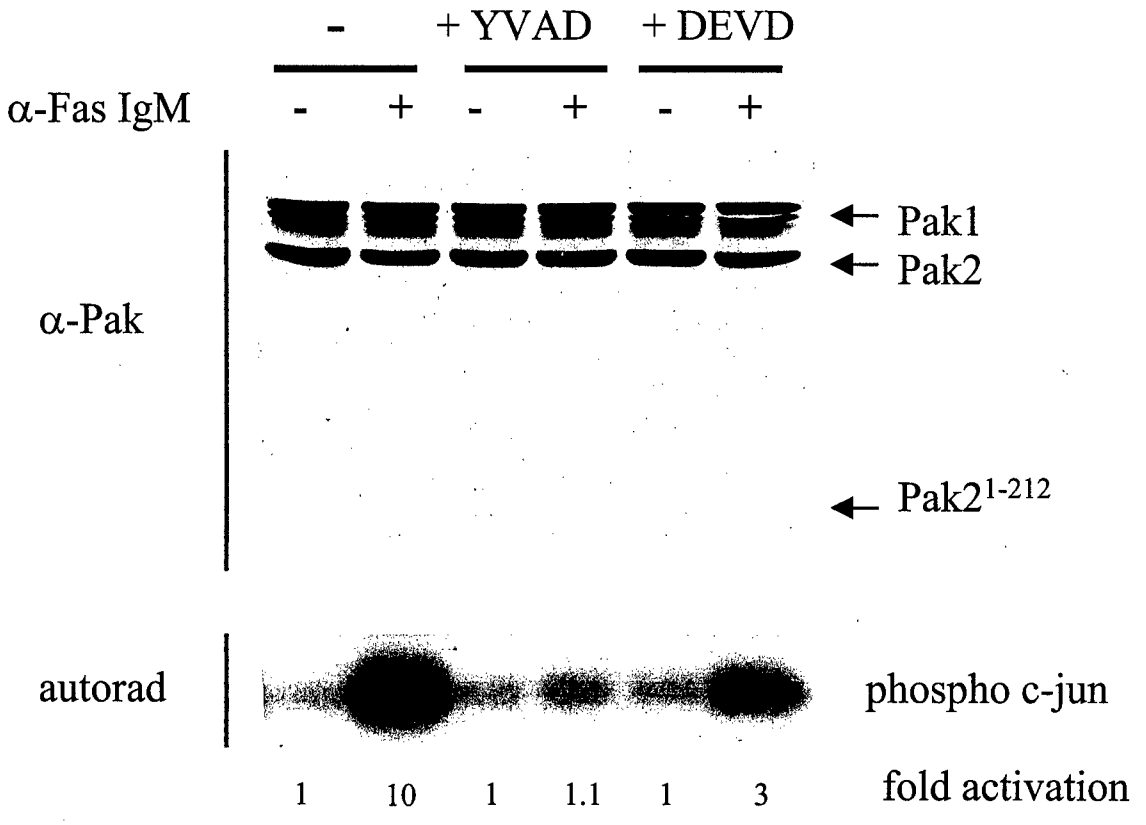

Figure 1B: **Inhibition of Fas-induced Pak2 cleavage and JNK activation by caspase inhibitors.** T47-D cells were stimulated with anti-Fas IgM or 6 hours. Cells were lysed and analyzed for Pak2 cleavage by immunoblotting and JNK activation by phosphorylation of GST-cjun (lower panel). YVAD-cmk and DEVD-ald were used at 100 μM and preincubated for 1 hour before Fas-ligation. Note the presence of the N-terminal Pak2 cleavage product in Fas-stimulated control cells (lane 2) and DEVD-treated cells (lane 6). GST-cjun phosphorylation was visualized by autoradiography and quantified by phosphorimaging analysis.

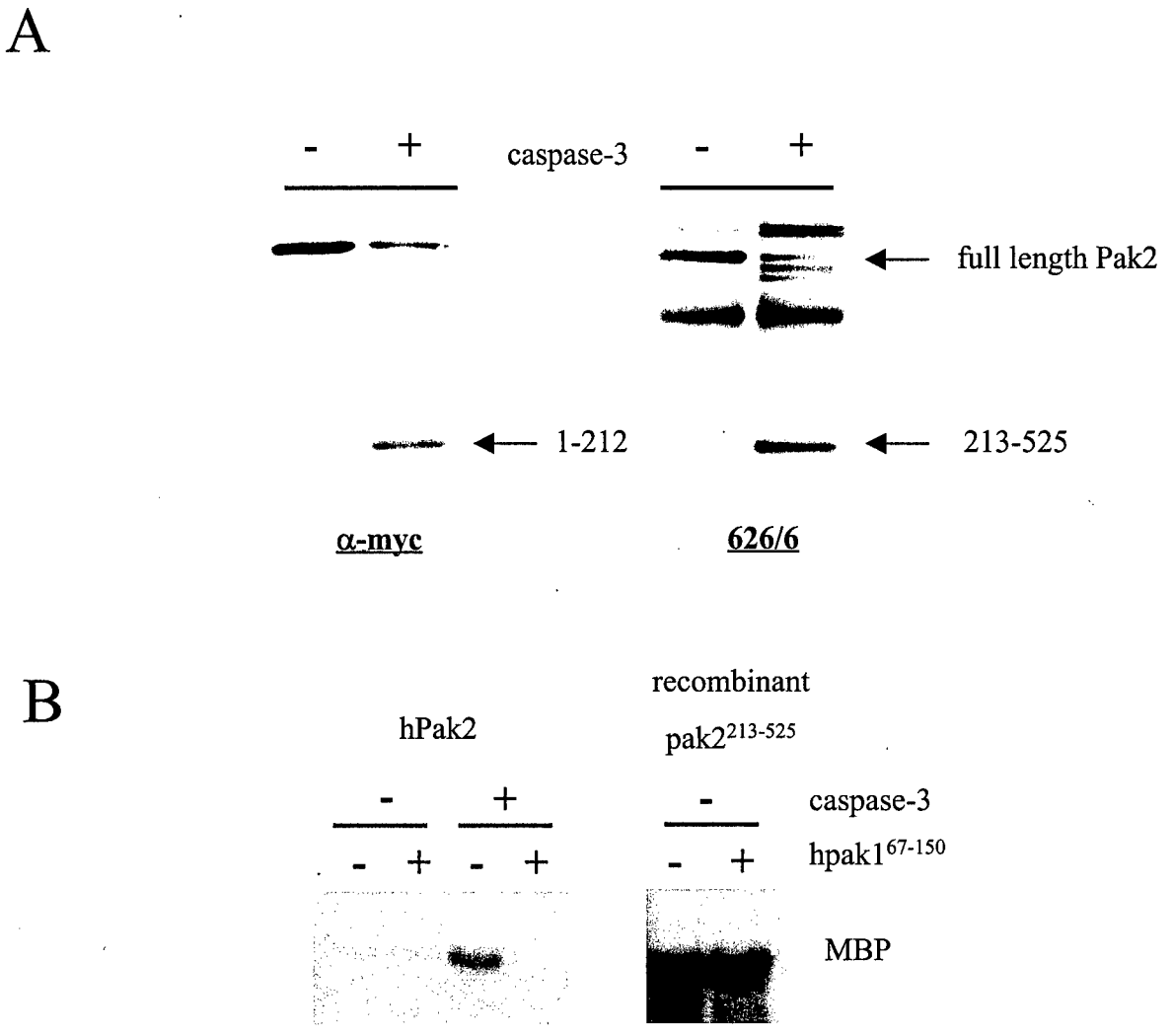

Figure 2: **Caspase-3 cleavage of human Pak2 and activation of kinase activity.** Human Pak2 was expressed in Cos-1 cells and immunoprecipitated with the monoclonal α-myc antibody. The immunoprecipitates were incubated with bacterially expressed caspase-3 for 1.5 hours at 37°C as indicated. A) The reactions were separated by SDS-PAGE and immunoblotted using the α-myc (9E10) and Pak-specific antiserum (626/6). The N-terminal (1-212) and C-terminal (213-525) cleavage products of Pak2 are labeled. B) Untreated-, caspase-3 treated Pak2 and recombinantly expressed His-pak2$^{213-525}$ were tested in *in vitro* kinase assays using myelin basic protein as a substrate. The recombinant inhibitory peptide (hpak1$^{67-150}$) was added to kinase reactions at 4 μM concentration. Nonlabeled ATP was used at 250 μM concentration, Labeled ATP at 10 μCi/reaction, MBP at 4 μg/reaction. Kinase reactions were separated by SDS-PAGE, and the dried gels were subjected to autoradiography.

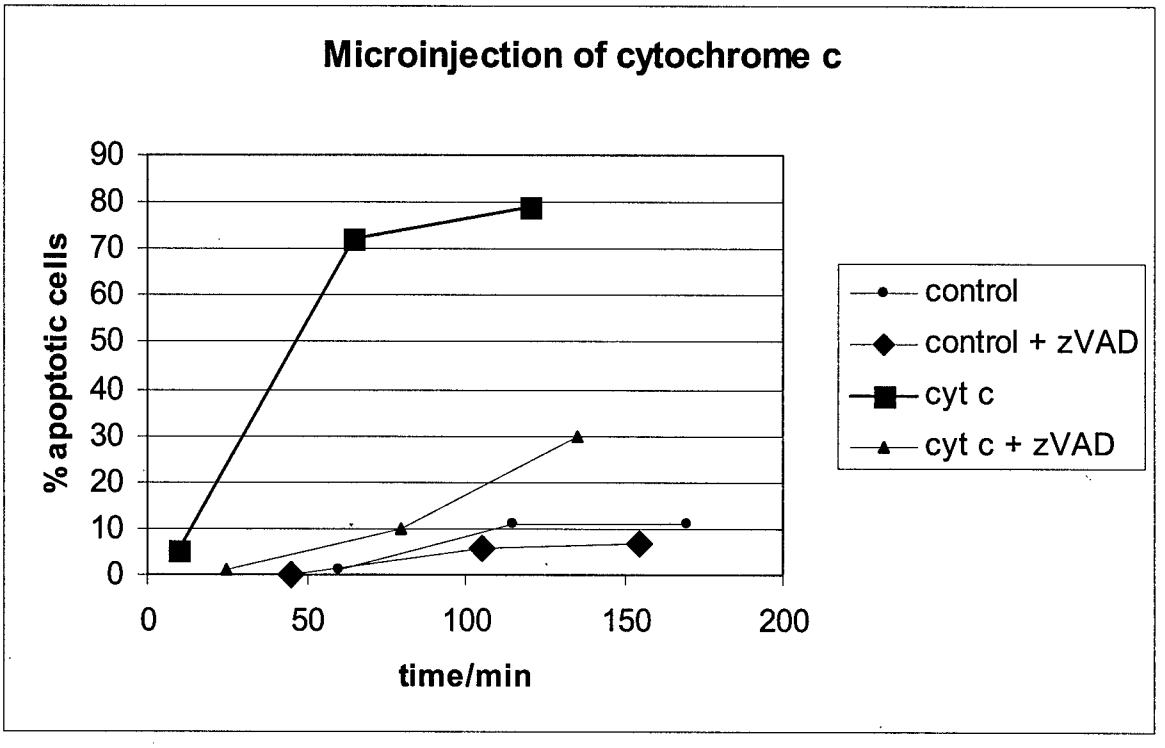

Figure 3: **Induction of apoptosis by cytochrome c injection into Swiss3T3 fibroblasts.** Cells were plated in 35 mm dishes and grown o/n. Microinjection was performed using an Eppendorf Micromanipulator 5171. About 100 cells were injected with Tetramethylrhodamine (TMR) as a fluorescent marker alone or in combination with cytochrome c (3 mg/ml) and/or zVAD-fmk (1 mM). Concentration refer to conditions in injection buffer (Tris/HCl pH7.5, 50 mM NaCl). Microinjected cells were identified by Rhodamine fluorescence and scored for apoptotic morphology at the indicated time points.

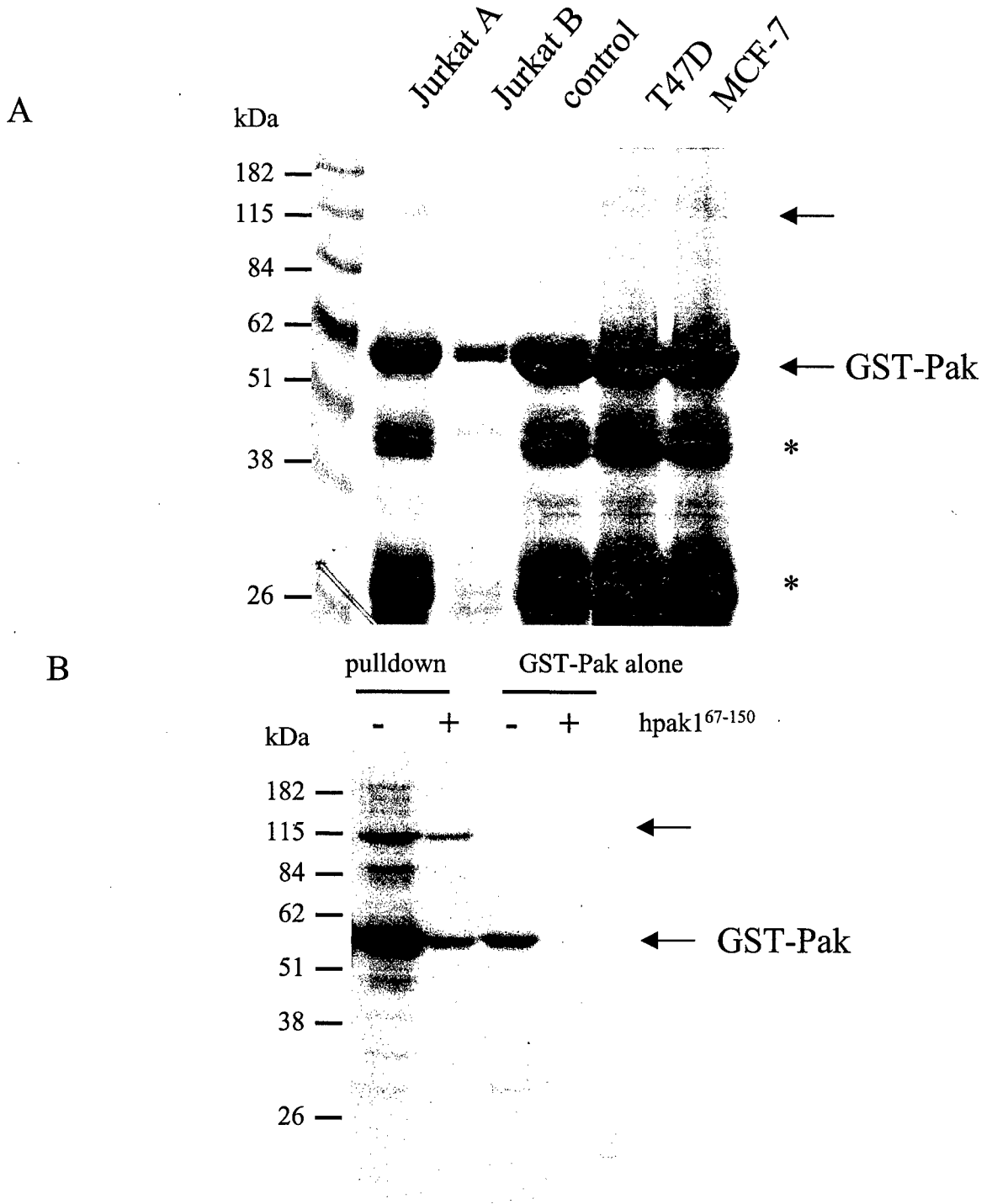

Figure 4: **Binding of a 115 kDa protein to GST-Pak.** A) Lysates from Jurkat T cells, T47-D and MCF-7 cells were incubated with immobilized GST-Pak1 (amino acids 233-544) for 1 hour at 4°C. The binding reactions were washed extensively and subjected to SDS-PAGE. As control GST-Pak1 alone was loaded (lane3). Note the presence of a 115 kDa protein especially in pulldowns from Jurkat lysates (arrow). Asterisks mark GST-Pak1 degradation products. B) GST-Pak1 pulldown fractios were subjected to an *in vitro* kinase assay. Equivalent amounts of the pulldown fraction and GST-Pak1 alone were incubated for 30 min at 30°C in the presence of 25 μM ATP and 10 μCi radiolabelled ATP. The inhibitory peptide (hpak1[1]) was added at 4 μM concentration as indicated. Kinase reactions were electrophoresed on a 10% SDS-PAA gel, the gel was dried and subjected to autoradiography. The 115 kDa protein and GST-Pak1 are marked.

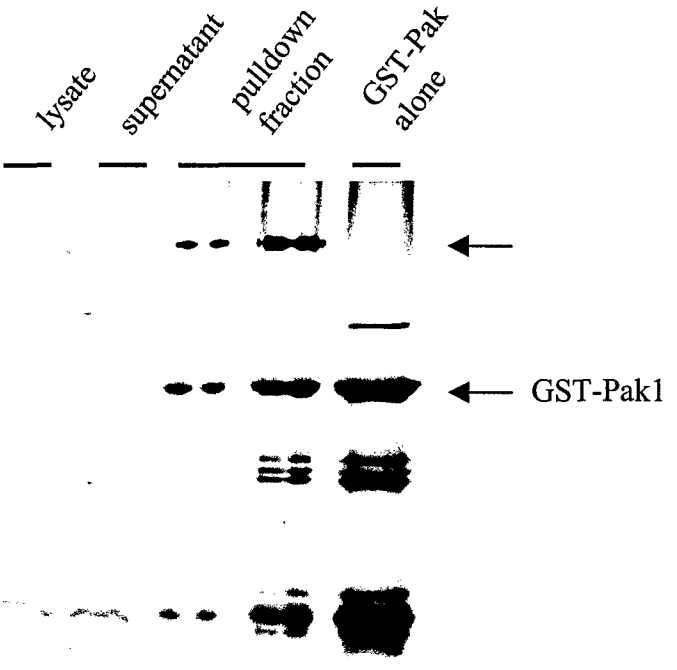

Figure 5: **Detection of the 115 kDa protein using *a*-GEF-H1 antibodies.** GST-Pak1 was immobilized on glutathione agarose and incubated with Jurkat lysate for 1 hour at 4°C. Jurkat lysate, the supernatant of the binding reaction, the pulldown fraction and GST-Pak1 alone were electrophoresed on 10% SDS-polyacrylamide gels and imunoblotted using antibodies raised against GEF-H1 (Ren et al., 1998). The pulldown fraction was loaded at two different concentrations. The 115 kDa protein and GST-Pak1 are labeled. Note that due to the use of a GST-GEF-H1 fusion protein for antiserum production, antibodies reacting against GST are present in the antiserum and detect GST-Pak1.

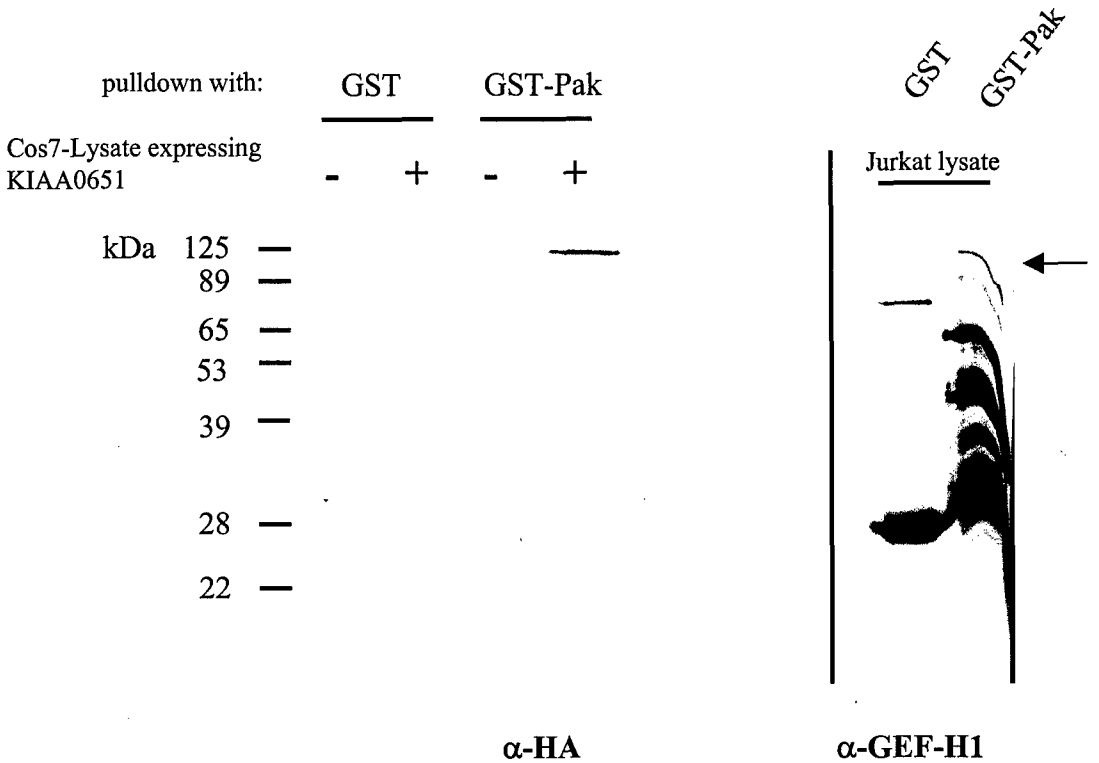

Figure 6: **Interaction between GST-Pak1 and KIAA0651.** KIAA0651 (+) and a control vector (-) were expressed in Cos7 cells and the resulting lysates were used in binding assays. GST or GST-Pak1 immobilized on glutathione agarose beads were incubated with control lysate, KIAA0651 containing lysate or Jurkat lysate for 1 hour at 4°C, washed four times with lysis buffer and subjected to SDS-PAGE. Western blotting was performed using anti-HA (12CA5) and α-GEF-H1 polyclonal antiserum. Note that the expressed epitope-tagged KIAA0651 is of similar mobility as the protein precipitated from Jurkat lysate (right panel, arrow1).

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
504 SCOTT STREET
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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 21 Feb 03

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

FOR THE COMMANDER:

Encl

PHYLLIS M. RINEHART
Deputy Chief of Staff for
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